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The overall goal of this research project was to investigate the cellular and membrane mechanisms associated with heterosynaptic modulation of long-term synaptic potentiation (LTP) at mossy fiber synapses in the hippocampus. Previous work in this laboratory had shown that norepinephrine (NE), through β-adrenoceptors, enhances the magnitude, duration, and probability of induction of mossy fiber LTP. We also had preliminary evidence that acetylcholine (ACh), through muscarinic receptors, depresses the magnitude and probability of induction of mossy fiber LTP. We hypothesized that the heterosynaptic modulation of mossy fiber LTP was through modulation of voltage-gated calcium channels. That is, the modulation of LTP by NE results from an enhancement of voltage-gated calcium channels, while suppression of LTP by ACh was through a decrease in activity of voltage-gated calcium channels. This hypothesis, and a number of assumptions associated with it, was tested during the past three years of this grant. Our results strongly support this hypothesis as well as the more general hypothesis that voltage-gated calcium channels are involved in the induction of mossy fiber LTP.

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22a. NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Dr. Genevieve M Haddad	(202) 767-5021	NL

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Final Technical Report

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1 Summary

The overall goal of this research project was to investigate the cellular and membrane mechanisms associated with heterosynaptic modulation of long-term synaptic potentiation (LTP) at mossy fiber synapses in the hippocampus. Previous work in this laboratory had shown that norepinephrine (NE), through β-adrenoceptors, enhances the magnitude, duration, and probability of induction of mossy fiber LTP. We also had preliminary evidence that acetylcholine (ACh), through muscarinic receptors, depresses the magnitude and probability of induction of mossy fiber LTP. We hypothesized that the heterosynaptic modulation of mossy fiber LTP was through modulation of voltage-gated calcium channels. That is, the modulation of LTP by NE results from an enhancement of voltage-gated calcium channels, while suppression of LTP by ACh was through a decrease in activity of voltage-gated calcium channels. This hypothesis, and a number of assumptions associated with it, was tested during the past three years of this grant. Our results strongly support this hypothesis as well as the more general hypothesis that voltage-gated calcium channels are involved in the induction of mossy fiber LTP.

We feel that we have accomplished many of the originally proposed objectives, as well as pursuing additional avenues of research that were not foreseen at the beginning of the project period. For the first two years of this project, we also had a collaboration with Dr. David Terrian, who was then at USAFSAM in San Antonio. Dr. Terrian subsequently moved to East Carolina University, and was not a part of this grant during the third year of funding. We feel that we have made significant progress in a number of directions, all of which are associated with our attempt to understand the mechanisms of synaptic plasticity in the mammalian central nervous system and the neuromodulation of synaptic plasticity by specific neurotransmitters.

2 Research Objectives

The research objectives for the funding period of 1 April 1988 to 31 March 1991 were as follows:

a) Test the hypothesis that the membrane potential of the postsynaptic (CA3) neuron is a variable for the induction of mossy fiber LTP. This hypothesis assumes that the induction of mossy fiber LTP follows a Hebbian rule.

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b) Test the hypothesis that a rise in intracellular calcium concentration in the postsynaptic neuron is required for the induction of mossy fiber LTP.

c) Test a number of hypotheses related to the heterosynaptic modulation of mossy fiber synapses and LTP by NE and ACh.

d) Conduct pilot experiments to test the hypothesis that serotonin (5-HT) is also involved in the heterosynaptic modulation of LTP.

e) Test the hypothesis that multiple types of voltage-gated calcium channels are present in cell bodies and dendrites of hippocampal neurons.

f) Test the hypothesis that there are different types of voltage-gated calcium channels in different classes of hippocampal neurons.

g) Test the hypothesis that voltage-gated calcium channels are modulated by norepinephrine and acetylcholine.

h) Develop a preparation of acutely isolated mossy fiber synaptic terminals and test the hypothesis that there are unique types of voltage-gated calcium channels in presynaptic terminals.

i) Develop a single neuron computer model for simulating postsynaptic calcium gradients as a substrate for the induction of LTP.

j) During years 1 and 2, Dr. David Terrian developed a subcellular fraction of mossy fiber synaptic terminals and, using biochemical techniques, tested hypotheses for the regulation of transmitter release by voltage-gated calcium channels, excitatory amino acids, and specific neuromodulators.

3 Status of Research

3.1 Test the hypothesis that the membrane potential of postsynaptic CA3 neurons is a variable for the induction of mossy fiber LTP; and

3.2 Test the hypothesis that a rise in intracellular calcium concentration in the postsynaptic neuron is required for the induction of mossy fiber LTP.

At most synapses in the hippocampus, activation of NMDA-type glutamate channels is required for the induction of LTP (Collingridge, Kehl and McLennan 1983). It has been hypothesized that calcium influx through these NMDA channels is the requisite first step in the induction of LTP (MacDermott et al. 1986; Malenka et al. 1988). In contrast, LTP at mossy fiber synapses is *independent* of the activation of NMDA receptors (Harris and Cotman 1986; Williams and Johnston 1988), and there is a very low density of NMDA receptors in the vicinity of these synapses (Monaghan and Cotman 1985). It is obvious, therefore, that some other mechanism must be involved in the induction of mossy fiber LTP. We have proposed, based on previous work, that activation of postsynaptic voltage-gated calcium channels by the high frequency stimulation normally used to induce LTP (and the subsequent calcium entry through these calcium channels) provides an alternate mechanism for LTP at mossy fiber synapses (Hopkins and Johnston 1988). During the first year of this research project, we tested one aspect of this hypothesis by demonstrating that the induction of mossy fiber LTP was blocked by the postsynaptic injection of the calcium chelators BAPTA and QUIN-2 (Williams and Johnston 1989).

The second prediction of this calcium channel hypothesis for the induction of LTP is that the membrane potential of the postsynaptic neuron during high frequency stimulation of the mossy

fibers should be a variable for the induction of LTP. In other words, hyperpolarization below threshold for activation of voltage-gated calcium channels during high frequency stimulation should block the induction of LTP. Furthermore, depolarization during high frequency stimulation should enhance the magnitude and probability of induction of LTP. During the past two years, we performed such experiments, and the results support the hypothesis. We found that hyperpolarization blocked mossy fiber LTP, and depolarization enhanced LTP. The results suggest that mossy fiber LTP follows a Hebbian rule for induction—concurrent pre- and postsynaptic activity are required for the plasticity to occur. This work has been published (Jaffe and Johnston 1990).

3.3 Test a number of hypotheses related to the heterosynaptic modulation of mossy fiber synapses and LTP by NE and ACh.

We have explored the modulation of mossy fiber synaptic transmission by muscarinic agonists. We found that muscarinic agonists at low concentrations have little effect on synaptic transmission but block the induction of mossy fiber LTP. At higher concentrations, however, muscarinic agonists decrease synaptic transmission in the absence of any high frequency stimulation. We suggest that the effects of higher concentrations of muscarine occur through a presynaptic action and decrease the release of transmitters from mossy fiber synapses. These results have been published (Williams and Johnston, 1990).

We have also been exploring the heterosynaptic modulation of LTP at other synapses in the hippocampus. Interestingly, we have found that muscarinic agonists appear to enhance LTP at the Schaffer collateral synapse. This is extremely interesting as it suggests that ACh can have multiple and opposite effects on synaptic plasticity in different regions of the hippocampus.

3.4 Conduct pilot experiments to test the hypothesis that serotonin (5-HT) is also involved in the heterosynaptic modulation of LTP.

This work has only recently begun, but the results are quite interesting. We began by studying another synapse into the CA3 region, the commissural/associational synapse. Preliminary data suggest that 5-HT, when applied during high frequency stimulation, blocks the induction of LTP. This inhibition of LTP appears to be at concentrations of 5-HT well below those that modulate synaptic transmission. The mechanism of 5-HT in blocking LTP is currently being investigated. One hypothesis is that 5-HT hyperpolarizes neurons, as has been shown previously, and thus reduces the probability of induction of LTP. Another interesting possibility that we will be pursuing is that the 5-HT blockade of the induction of LTP involves decreases in cyclic AMP.

3.5 Test the hypothesis that multiple types of voltage-gated calcium channels are present in cell bodies and dendrites of hippocampal neurons; and

3.6 Test the hypothesis that there are different types of voltage-gated calcium channels in different classes of hippocampal neurons.

It has been reported in a number of preparations that at least three types of voltage-gated calcium channels exist in neurons (Miller 1987). In our studies of voltage-gated calcium channels in granule cells, we obtained preliminary evidence that there were three types (T, N, and L) (Gray and Johnston 1986). We wanted to test the hypothesis that these three types of calcium channels were present in CA1 and CA3 pyramidal neurons, to explore the distinguishing characteristics of the three types of channels, and to determine the relative distribution of these channels among the different hippocampal neurons. Figures 1-3 illustrate the differences in the three types of calcium channels observed in hippocampal neurons. The T channel has the smallest single channel conductance,

it inactivates rapidly with depolarization, and it has the lowest threshold for activation. The L channel, in turn, has the largest single channel conductance, it inactivates relatively little with prolonged depolarization, and it has the highest threshold for activation. The N channel has characteristics that fall in the middle of the T and L. We also found that there was a heterogeneous distribution of the three channel types among hippocampal neurons (see Fig. 5). For example, granule cells contain mostly N-type channels, CA1 neurons contain N- and L-type but fewer T-type than CA3, while all three channel types are abundant on CA3 neurons. The different distribution of channel types among the neurons may help explain their different functional properties. For example, the T-type channel has been suggested to underlie endogenous burst behavior in neurons (Llinás 1988) and the relative burst tendency of hippocampal neurons certainly follows our observed distribution of T-type channels (*i.e.*, granule cells < CA1 < CA3). This work on the properties of calcium channels in the hippocampus has been published (Fisher, Gray and Johnston 1990).

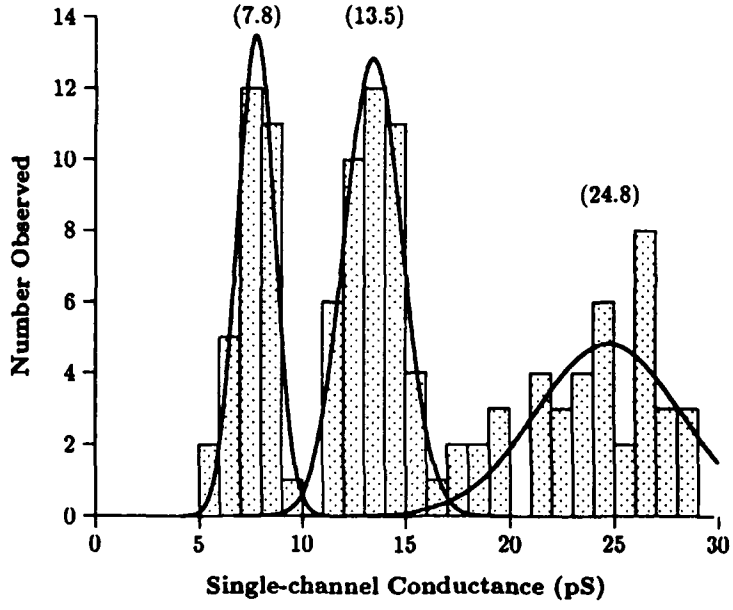

Figure 1: Frequency distribution of 115 single-channel conductances pooled from CA3 and CA1 pyramidal neurons and dentate granule cells. The distribution was fit simultaneously with the sum of three Gaussian curves. The mean value (and standard deviation) for each peak was 7.8 (0.9), 13.5 (1.4), and 24.8 pS (3.4). Binwidth of the distribution was 1 pS.

3.7 Test the hypothesis that voltage-gated calcium channels are modulated by norepinephrine and acetylcholine.

With the characterization of at least three types of calcium channels in CA3 pyramidal neurons (see above), it became feasible to test the hypothesis that norepinephrine, through β-adrenoceptors, modulates specific types of calcium channels. Briefly, we found that isoproterenol enhanced the activity of the N- and the L-type channels with essentially no effect on the T-type channel. A summary of the results are illustrated in Figure 6. These results have been published (Fisher and Johnston 1990).

An obvious hypothesis that we derived from our finding that muscarine depresses mossy fiber LTP is that muscarinic agonists might depress voltage-gated calcium channels. We have investigated the effects of carbachol and muscarine on the three types of voltage-gated calcium channels observed in hippocampus. The results are quite interesting. We found that muscarinic agonists depress the

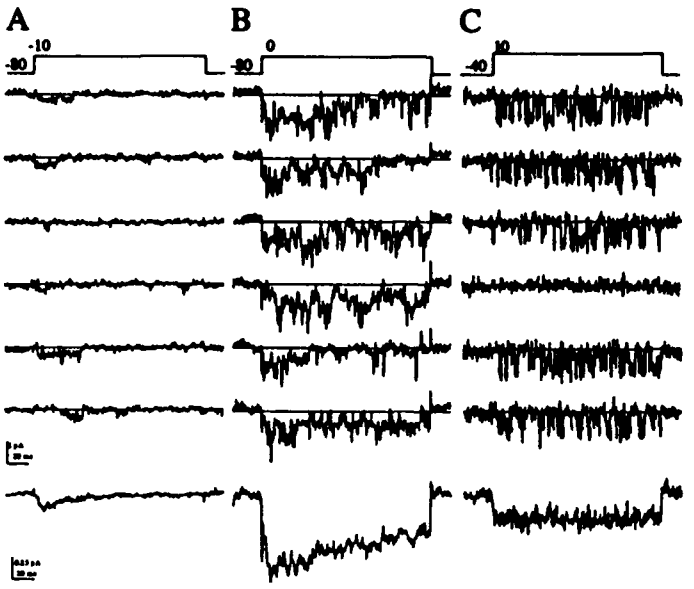

Figure 2: Ensemble averages reveal differences in inactivation of the three types of channels. Single-channel records and ensemble averages from three different patches. **A.** traces from a patch that contained only the 8 pS channel, showing complete channel inactivation during the command step (average of 128 traces). **B.** a patch that contained only 14 pS channels shows considerable inactivation during the command step (average of 41 traces). **C.** a patch that contained only 25 pS channels shows no significant inactivation during the command step (average of 77 traces).

L-type, have no effect on the N-type, and increase the T-type calcium channel (see summary in Fig. 7). These results have been published (Fisher and Johnston 1990). One interesting speculation derived from these results, and those mentioned above, is that the L-type calcium channel may be involved in the induction of mossy fiber LTP.

3.8 Develop a preparation of acutely-isolated, mossy fiber synaptic terminals and test the hypothesis that there are unique types of voltage-gated calcium channels in presynaptic terminals.

During this grant period, we have continued our investigation of presynaptic calcium channels at mossy fiber synapses. Using cell attached patch recordings, we have recorded single voltage-gated calcium channels from mossy fiber presynaptic terminals. There appears to be multiple types of channels present in the terminals based on a wide range of single channel conductances recorded (see Fig. 4). None of the recorded channels, however, were affected by BAY K 8644 and are unlikely to be of the L-type.

Richard Gray, a co-investigator on this project, has begun using calcium imaging techniques for the visualization of intracellular calcium from the isolated mossy fiber terminals and has demonstrated a K stimulated increase in intraterminal calcium. This work was begun in collaboration with John Connor and is in progress. Rick has also successfully measured whole-cell calcium currents from presynaptic terminals using nystatin and the perforated patch technique (Korn and Horn 1989).

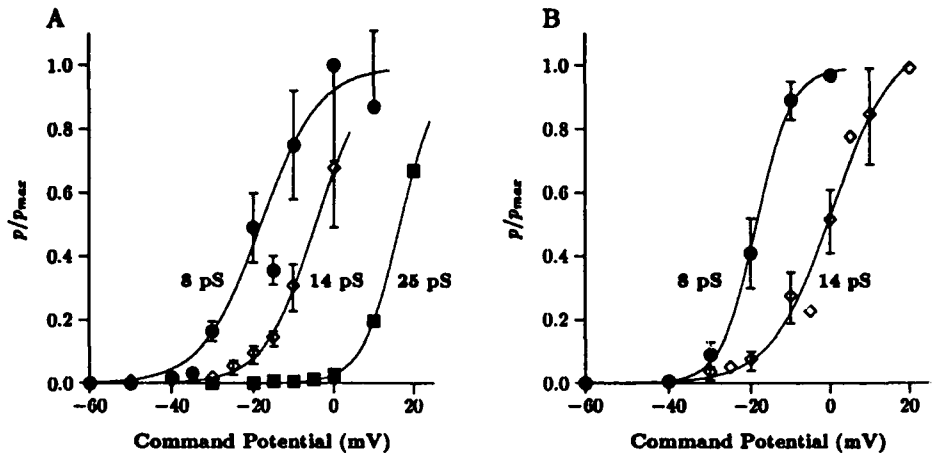

Figure 3: The three channel types show differences in voltage range of activation. p = probability of channel being in the open state (fractional open time) during the command step; p_{max} = maximal probability of channel opening; curve is a fitted sigmoid (for details, see METHODS). For the 8 and 14 pS channel data, the patches were held at −80 mV and stepped to the indicated command potentials. For the 25 pS curve, patches were held at −40 mV. **A.** Activation curves derived from patches from CA3 neurons. $V_{1/2}$ (half-activation points) and k (steepness) for each of the curves are: −18 mV, 7.2 (8 pS); −4 mV, 6.3 (14 pS); and 17 mV, 4.7 (25 pS). Each curve is the normalized average of several experiments: 10, 8, and 3 for the 8, 14, and 25 pS channels, respectively. **B.** Activation curve for the 8 pS channel derived from data from 2 granule and 2 CA1 neurons, and for the 14 pS channel from 3 granule neurons. For the 8 pS curve, $V_{1/2} = -19$ mV, $k = 4.3$; for the 14 pS curve, $V_{1/2} = 0$ mV, $k = 7.7$.

3.9 Develop a single neuron computer model for simulating postsynaptic calcium gradients through voltage-gated calcium channels as a substrate for the induction of LTP.

Although not part of the original grant application, we have continued to develop and use single neuron computer models to test various hypotheses related to mossy fiber synaptic transmission and LTP. Using our recent data for different types of calcium channels, we have constructed a fairly realistic model of CA3 pyramidal neurons. We are interested in the calcium influx through calcium channels during synaptic activity and the buffering of this calcium in different regions of the neuron (*e.g.* , spine head, spine shaft, and apical dendrite).

3.10 During years 1 and 2, Dr. David Terrian developed a subcellular fraction of mossy fiber synaptic terminals and, using biochemical techniques, tested hypotheses for the regulation of transmitter release by voltage-gated calcium channels, excitatory amino acids, and specific neuromodulators.

During the first two years of this project, we characterized the biochemical properties of isolated hippocampal mossy fiber synaptosomes and continued our investigation of the autoregulation of neurotransmitter release from these large nerve endings. Four specific hypotheses were tested concerning the biochemical basis for mossy fiber synaptic transmission and its presynaptic modulation.

1. The first hypothesis was that the excitatory mossy fiber synaptic input is mediated by both opioids and acidic amino acids. We had previously demonstrated that depolarized mossy fiber synaptosomes release both dynorphin B and endogenous glutamate in a Ca^{2+}-dependent man-

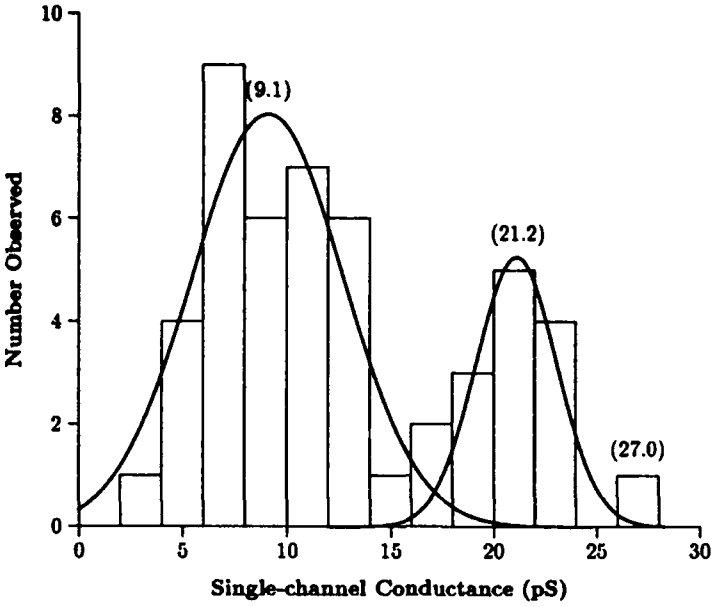

Figure 4: Distribution of slope conductances calculated from I–V plots made from cell-attached patch recordings from mossy fiber presynaptic terminals. The solid curves are the individual Gaussian distributions that best fit the data. Mean values are indicated above each peak. Binwidth was 2 pS.

ner (Terrian et al., 1988). However, the biochemical identity of the mossy fiber neurotransmitter had not been resolved and aspartate, in particular, was considered to be a legitimate candidate. Therefore, experiments were conducted to determine what relative amounts of prodynorphin-derived peptides and endogenous amino acids are concomitantly released from hippocampal mossy fiber synaptosomes.

2. The second hypothesis was that a presynaptic excitatory amino acid receptor autoregulates the release of neurotransmitters from mossy fiber terminals. Preliminary evidence to support this hypothesis was reported in our previous Annual Technical Report, where it was demonstrated that the glutamate analogue L(+)-2-amino-4-phosphonobutyric acid (APB) suppressed the evoked release of both glutamate and dynorphin from mossy fiber synaptosomes. During the present reporting period additional experiments were conducted to examine the mechanism of this presynaptic autoregulation and to identify the type of receptor that is involved.

3. The third hypothesis was that a presynaptic opioid autoreceptor also modulates the release of mossy fiber neurotransmitters. Immunocytochemical and autoradiographic studies had previously been able to provide indirect support for such a presynaptic receptor, but no direct biochemical evidence was available.

4. The fourth hypothesis was that the presynaptic mechanism(s) underlying the maintenance of LTP in the mossy fiber synapse does *not* involve the activation of protein kinase C. It has been proposed that the maintenance of LTP in the mossy fiber synapse involves fundamentally different presynaptic mechanisms from those employed by other hippocampal synapses, since both protein kinase C and phosphoprotein F1 (GAP-43) appear to be absent in mossy fiber terminals. Using the mossy fiber synaptosomal preparation, it was possible to directly test this hypothesis.

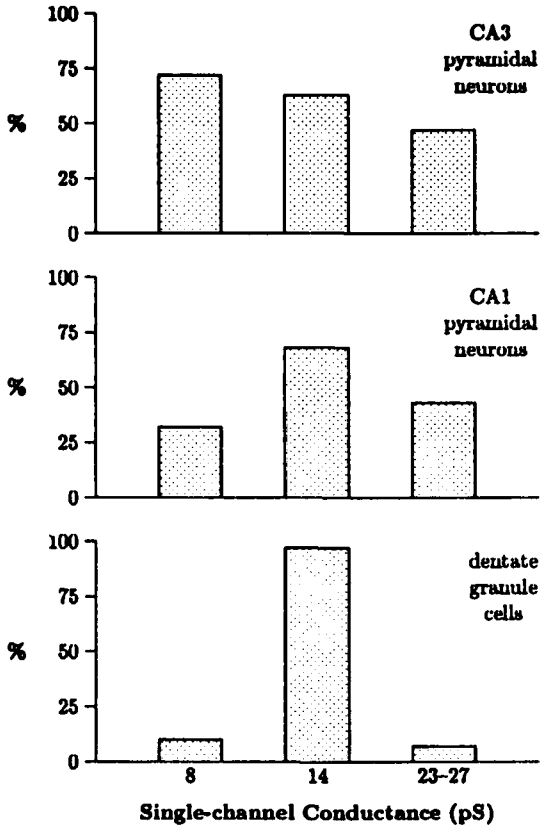

Figure 5: Relative distribution of the three channel types in each of the three principal cell types of the hippocampal formation. Each channel measured was classified as small (8 pS), medium (14 pS), or large (25 pS) conductance and binned accordingly. **A.** CA3 neurons. Distribution of small and medium-conductance channels taken from 32 patches; distribution of large-conductance channel taken from 64 patches. **B.** CA1 neurons. Distribution taken from 28 patches. **C.** granule cells. Distribution from 29 patches.

The results of the experiments that were conducted during the second year to test the first two hypotheses have been published (Gannon, Baty and Terrian 1989; Gannon and Terrian 1989; Gannon and Terrian 1990; Terrian, Dorman and Gannon 1990; Terrian, Gannon and Rea 1990). The later two hypotheses were tested by Dr. Terrian more recently, following his relocation to the East Carolina University School of Medicine. The results of these experiments can be summarized as follows:

1. Of the 18 amino acids shown to be present in superfusate fractions by liquid chromatographic analysis, only glutamate was released at a significantly enhanced rate from depolarized mossy fiber nerve endings. The release of glutamate and aspartate was increased by 360 ± 27% and 54 ± 12% over baseline, respectively. However, the evoked release of glutamate was substantially more Ca^{2+}-dependent (80%) than was the release of aspartate (49%). Depolarization also stimulated the release of the four prodynorphin (Dyn) products examined, in a rank order of Dyn B >> Dyn A(1–17) > Dyn A(1–8) >> Dyn A(1–13), with Dyn B efflux increasing by more than 5-fold over baseline values. These results suggest that the predominant excitatory amino acid in hippocampal mossy fiber synaptic transmission may be glutamate and that this synaptic input may be modulated by at least four different products of prodynorphin processing.

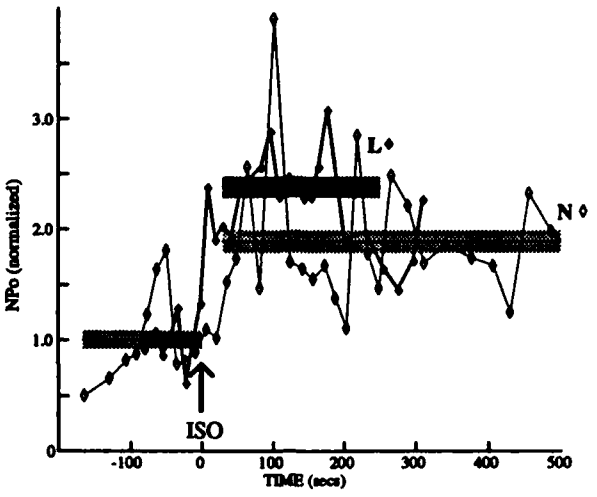

Figure 6: Summary of isoproterenol effects on all patches containing N and L channels. Filled diamonds represent averaged data points for all experiments on L channels; darkly shaded boxes are mean ± sem before and after isoproterenol application to L channels. Unfilled diamonds represent averaged points for all experiments on N channels; lightly shaded boxes are mean ± sem before and after isoproterenol application to N channels. Average changes in NP_o were as follows: 89% increase (Ns, 7 experiments) and 138% increase (Ls, 6 experiments). Plots of probability of channel opening *vs* time for each individual experiment were normalized for both x and y axis values (so that, for each experiment, drug application occurred at time = 0 seconds and the average pre-drug value of NP_o was 1.0). All normalized data points for all experiments on N (or L) channels were then pooled. Every 30 data points were averaged and plotted as one point in this figure.

2. Excitatory amino acid agonists and antagonists were evaluated for their ability to affect the concomitant release of endogenous glutamate and Dyn A(1–8) from guinea pig mossy fiber synaptosomes. Low micromolar concentrations of quisqualate, but not kainate, *N*-methyl-D-aspartate (NMDA), nor RS-alpha-amino-3-hydroxy-5-methyl-4-isoazole-propionic acid, significantly inhibited the depolarization-evoked release of both glutamate and Dyn A(1–8). Quisqualate-induced inhibition of glutamate release from mossy fiber terminals was antagonized by the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). In contrast, high concentrations of kainate enhanced the evoked release of glutamate and Dyn A(1–8), and this potentiation was blocked by CNQX. These results suggest a bimodal mechanism for the autoregulation of neurotransmitter release from mossy fiber terminals. Previous reports have indicated that the presynaptic kainate receptors may contribute to the unusual sensitivity of the mossy fiber-CA3 pathway to epileptic damage and that these receptors may only become fully expressed or active during the reactive synaptogenesis that occurs following hippocampal neuronal damage.

3. We have recently examined the effects of the κ agonist U50488H on calcium availability and the release of glutamate and Dyn B from mossy fiber synaptosomes. The results of these studies demonstrated that U50488H produces a dose-dependent inhibition of the depolarization-induced rise in cytosolic free calcium and the release of both neurotransmitters. The estimated IC_{50} value for this effect is 30 μM. The inhibitory effect of U50488H was also reversed by the selective κ antagonist nor-binaltorphimine. These results suggest the existence of a κ opioid

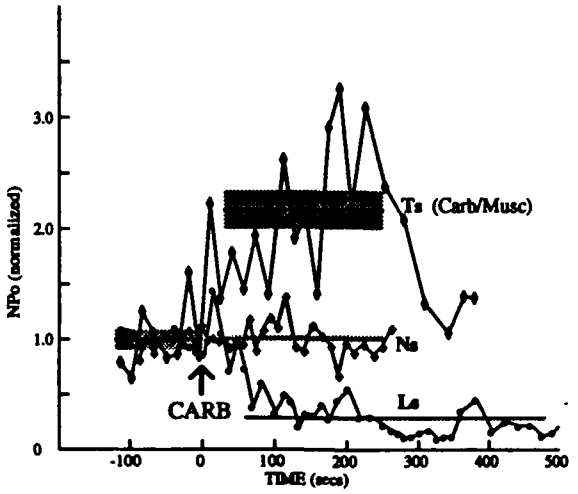

Figure 7: Summary of carbachol effects on all patches containing T and L channels. Unfilled diamonds represent averaged data points for all experiments on T channels, filled diamonds for N channels, and filled circles for L channels. Shaded boxes are mean ± sem before and after carbachol application. The post-drug variation for N and L channels was too small for the shaded boxes to be clearly visible. The pre-drug shaded box is for carbachol on T channels; the corresponding values for N and L channels showed less variability. Average changes in NP_o were as follows: 117 % increase (Ts, 6 experiments), 1 % increase (Ns, 9 experiments), and 71 % decrease (Ls, 7 experiments). The plot for T channels includes both carbachol and muscarine data. Normalization and plotting of data are the same as described in Fig. 5 legend.

autoreceptor capable of modulating mossy fiber synaptic transmission.

4. The metabolic properties of hippocampal mossy fiber synaptosomes were compared to those of a conventional P_2 synaptosomal fraction prepared from the same hippocampal tissue. Protein kinase C-dependent histone phosphotransferase activity was found to be comparable in mossy fiber and P_2 synaptosomes. Western blot analyses were performed to confirm this unexpected finding, and the results demonstrate that the α, β, and γ subspecies of protein kinase C are all present in relatively equivalent amounts in these two different subcellular fractions. However, an SDS-PAGE analysis of the endogenous substrates phosphorylated by protein kinase C indicated that protein F1 is not present in mossy fiber nerve endings. A functional role for protein kinase C in the mossy fiber terminal seems to be indicated by the finding that phorbol-12,13-dibutyrate and phorbol-12,13-diacetate produce a dose-dependent potentiation of the K^+-evoked increase in the availability of cytosolic free calcium and the concomitant release of endogenous glutamate and Dyn B. The biologically inactive 4-α-phorbol was without affect on any of these parameters, and the phorbol-12,13-dibutyrate (1 μM) enhancement of Ca^{2+}-dependent release was blocked by the protein kinase C antagonist staurosporine (1 μM). Based on these results, we conclude that hippocampal mossy fiber nerve endings possess a variety of protein kinase C isoforms and that their activation is sufficient to have an important influence on mossy fiber synaptic transmission and plasticity.

4 Publications

4.1 Full papers and review articles

1. Terrian, D.M., Johnston, D., Claiborne, B.J., Ansah-Yiadom, R., Strittmatter, W.J., and Rea, M.A. Glutamate and dynorphin release from a subcellular fraction enriched in hippocampal mossy fiber synaptosomes. *Brain Res. Bull.* 21:343-351, 1988.

2. Williams, S. and Johnston, D. Long-term potentiation of hippocampal mossy fiber synapses is blocked by postsynaptic injection of calcium chelators. *Neuron* 3:583–588, 1989.

3. Johnston, D., Hopkins, W.F., and Gray, R. The role of norepinephrine in long-term potentiation at mossy fiber synapses in the hippocampus. In: *Neural Models of Plasticity.* Byrne, J.H. and Berry, W.O. (*eds.*), Academic Press, Inc.: San Diego, 1989, pp. 307–328.

4. Hopkins, W.F. and Johnston, D. Noradrenergic modulation of synaptic plasticity in the hippocampus. In: *Problems and Concepts in Developmental Neurophysiology.* Kellaway, P. and Noebels, J.L., (*eds.*), Johns Hopkins Univ. Press: Baltimore, 1989, pp. 92–109.

5. Johnston, D., Williams, S.H., Gray, R, and Fisher, R.E. Cholinergic and noradrenergic modulation of long-term potentiation in hippocampal CA3 neurons. In: *Brain Signal Transduction and Memory.* Ito, M. and Nishizuka, Y. (*eds.*), Academic Press, Inc.: San Diego, 1989, pp. 171–183.

6. Fisher, R., Gray, R., and Johnston, D. Properties and distribution of single voltage-gated calcium channels in adult hippocampal neurons. *J. Neurophysiol.* 64:91–104, 1990.

7. Jaffe, D. and Johnston, D. The induction of long-term potentiation at hippocampal mossy fiber synapses follows a Hebbian rule. *J. Neurophysiol.* 64:948–960, 1990.

8. Rutecki, P.A., Lebeda, F.J., and Johnston, D. Epileptiform activity in the hippocampus produced by tetraethylammonium. *J. Neurophysiol.* 64:1077–1088, 1990.

9. Williams, S. and Johnston, D. Muscarinic depression of synaptic transmission at the hippocampal mossy fiber synapse. *J. Neurophysiol.* 64:1089–1097, 1990.

10. Fisher, R. and Johnston, D. Differential modulation of single voltage-gated calcium channels by cholinergic and adrenergic agonists in adult hippocampal neurons. *J. Neurophysiol.* 64:1291–1302, 1990.

11. Gray, R., Fisher, R.E., Spruston, N., and Johnston, D. Acutely exposed hippocampal neurons: A preparation for patch clamping neurons from adult hippocampal slices. In: *Preparations of Vertebrate Central Nervous System* In Vitro. Jahnsen, H. (*ed.*), John Wiley: England, 1990, pp. 3–24.

12. Chetkovich, D.M., Gray, R., Johnston, D., and Sweatt, J.D. NMDA-receptor activation increases cAMP levels and voltage-gated Ca^{2+}-channel activity in area CA1 of hippocampus. *Proc. Nat. Acad. Sci.* (USA), (in press).

13. Williams, S. and Johnston, D. Kinetic properties of two anatomically distinct excitatory synapses in hippocampal CA3 pyramidal neurons. *J. Neurophysiol.*, (in press).

14. Spruston, N. and Johnston, D. Perforated patch-clamp analysis of the passive membrane properties of three classes of hippocampal neurons. *J. Neurophysiol.*, (submitted).

15. Johnston, D., Fisher, R.E., and Gray, R. Voltage-gated calcium channels in adult hippocampal neurons. In: *Ion Channels, Volume III.* Narahashi, T., (*ed.*), Plenum Publishing Corp: New York, (in press).

16. Johnston, D., Williams, S., Jaffe, D., and Gray, R. NMDA-receptor independent long-term potentiation. *Annual Review of Physiol.*, Academic Press, Inc.: San Diego, (in press)

4.2 Abstracts

1. Johnston, D. and Williams, S.H. Muscarine depresses long-term potentiation in CA3 neurones of the rat hippocampus. *J. Physiol.* 407:49P, 1988.

2. Fisher, R.E., Gray, R., and Johnston, D. β-adrenoceptor modulation of calcium channels in acutely exposed CA3 pyramidal neurons of adult guinea pig hippocampus. *Soc. Neurosci. Abstr.* 14:645, 1988.

3. Gray, R. and Johnston, D. Recordings of single calcium channels from presynaptic mossy fiber terminals in adult guinea pig hippocampus. *Soc. Neurosci. Abstr.* 14:68, 1988.

4. Jaffe, D.B. and Johnston, D. Depression of synaptic transmission by ω-conotoxin in the rat hippocampal slice. *Soc. Neurosci. Abstr.* 14:810, 1988.

5. Williams, S.H. and Johnston, D. Muscarine depresses an APV-insensitive form of LTP in CA3 hippocampal neurons. *Soc. Neurosci. Abstr.* 14:564, 1988.

6. Williams, S.H. and Johnston, D. Hippocampal CA3 mossy fiber LTP is blocked by postsynaptic calcium chelators. *Soc. Neurosci. Abstr.* 15:399, 1989.

7. Jaffe, D.B. and Johnston, D. Voltage-dependence of LTP at the hippocampal mossy fiber synapse. *Soc. Neurosci. Abstr.* 15:399, 1989.

8. Jaffe, D.B. and Johnston, D. Modelling changes in $[Ca^{2+}]_i$ in hippocampal CA3 neuron by voltage-gated Ca^{2+} channels. *Soc. Neurosci. Abstr.* 16:58, 1990.

9. Schulz, P.E. and Johnston, D. Muscarinic modulation of rat hippocampal CA1 long-term potentiation (LTP). *Soc. Neurosci. Abstr.* 16:653, 1990.

10. Schulz, P. and Johnston, D. Dissociation of muscarinic effects on rat hippocampal CA1 and CA3 LTP. *Neurology* 40 (Suppl. 1):373, 1990.

11. Spruston, N. and Johnston, D. Whole-cell patch clamp analysis of the passive membrane properties of hippocampal neurons. *Soc. Neurosci. Abstr.* 16:1297, 1990.

12. Williams, S.H. and Johnston, D. An analysis of the muscarinic depression of synaptic transmission at the CA3 mossy fiber synapse. *Soc. Neurosci. Abstr.* 16:59, 1990.

13. Schulz, P.E. and Johnston, D. Muscarinic receptor-mediated enhancement of long-term potentiation (LTP) may involve increased depolarization during tetanus. *Neurology* 41(Suppl 1):388, 1991.

14. Schulz, P.E. and Johnston, D. Evidence for differing mechanisms of short- and long-term potentiation expression. *Soc. Neurosci. Abstr.* 17:(in press), 1991.

15. Williams, S.H. and Johnston, D. Kinetic analysis of synaptic currents at two excitatory inputs to the hippocampal CA3 pyramidal neuron. *IBRO*, 1991.

16. Williams, S.H. and Johnston, D. Comparison of two anatomically distinct excitatory synaptic inputs to CA3 pyramidal neurons. *Soc. Neurosci. Abstr.* 17:(in press), 1991.

17. Chetkovich, D.M., Gray, R., Johnston, D., and Sweatt, J.D. NMDA receptors cause Ca^{2+}-dependent increases in cAMP levels and Ca^{2+}-channel activity. *Soc. Neurosci. Abstr.* 17:(in press), 1991.

18. Villani, F. and Johnston, D. Modulation of the hippocampal commissural-CA3 long-term potentiation by serotonin. *Soc. Neurosci. Abstr.* 17:(in press), 1991.

19. Jaffe, D.B., Ross, W.N., and Johnston, D. The distribution of dendritic calcium entry produced by action potentials in a model hippocampal CA3 neuron. *Soc. Neurosci. Abstr.* 17:(in press), 1991.

20. Ross, W.N., Jaffe, D.B., Lasser-Ross, N., Lisman, J., and Johnston, D. Large increases in intracellular Ca^{2+} require postsynaptic action potentials in hippocampal pyramidal neurons. *Soc. Neurosci. Abstr.* 17:(in press), 1991.

5 Professional Personnel Associated With the Research Project

Daniel Johnston, Ph.D.—Principal Investigator
Richard Gray, Ph.D.—Research Assistant Professor
Flavio Villani, M.D.—Research Assistant
David Jaffe—Graduate Student
Nelson Spruston—Graduate Student
Judy Walker—Research Technician
Jennie Rexer—Research Technician
Mahmud Haque—Computer Systems Manager
David Terrian, Ph.D.—Co-investigator

6 Interactions

04/16/88– 04/21/88	Spring Hippocampal Research Conference, St. Thomas
04/16/88– 04/21/88	Mahmud Haque to Masscomp Users' Society Meeting in Boston
05/15/88– 05/17/88	Association of Neuroscience Departments and Programs Spring Meeting and Congressional Visits
06/01/88– 06/03/88	NIMH Study Section, Washington, DC
07/19/88	Spoke to students in Baylor's SMART Program
08/18/88	To Department of Physiology, University of Texas, Dallas
09/20/88	Gave seminar in Physiology Department, BCM
10/03/88	Gave lecture in Department of Physiology, UT, Dallas
10/12/88– 10/14/88	NIMH Study Section, Washington, DC
11/12/88– 11/16/88	Society for Neuroscience, Toronto, Canada
11/27/88– 11/30/88	5th Takeda Science Foundation on Bioscience, Kyoto, Japan
01/21/89– 01/26/89	Winter Conference on Brain Research, Snowbird, UT
02/22/89– 02/23/89	NIA site visit, Rockville, MD
03/06/89– 03/08/89	NIMH site visit, Portland, Oregon
03/29/89– 03/30/89	Gave lecture in Department of Biology, Brandeis University
4/8/89	Premedical Advisor Workshop
05/08/89– 05/14/89	Mahmud Haque to Masscomp User's Society, Boston, MA
6/23/89	Seminar for the Division of Neuroscience, Baylor. "Mechanisms of LTP in hippocampus."

06/29/89– 06/30/89	NIMH Study Section, Washington, DC
07/18/89– 07/19/89	Study Section "MHK Research Scientist Development," Washington, DC
08/04/89	Lecture for SMART students at Baylor
10/05/89	NIH–MBRS Symposium
10/29/89– 11/02/89	Society for Neuroscience, Phoenix, Arizona
12/10/89– 12/11/89	Study Section, Special Review Committee, Washington, D.C.
02/06/90	Lecture to MSTP students at Baylor
03/02/90	Gave seminar at the University of Texas Medical Branch in Galveston for the Department of Pharmacology. "NMDA independent LTP in hippocampus.
03/29/90– 03/30/90	Study Section, Special Review Committee, Washington, D.C.
04/03/90– 04/04/90	FASEB meeting, Washington, DC
04/21/90– 04/28/90	Gave two talks on mosy fiber synaptic transmission and epileptogenesis in the hippocampal slice at the Spring Hippocampal Research Conference, Grand Cayman Islands
06/02/90– 08/22/90	Collaborative research project with John Lisman and Bill Ross at the Marine Biological Laboratory, Woods Hole, MA
10/28/90– 11/02/90	Society for Neuroscience, St. Louis, MO
11/07/90– 11/08/90	Gave talk at the University of Chicago Neurobiology Department. "NMDA-independent LTP in hippocampus."
12/01/90– 12/02/90	Rush Record Neuroscience Forum, The Houstonian Hotel and Conference Center, Houston, TX
12/12/90– 12/14/90	Special Review Committee, Washington, DC
01/24/91– 01/26/91	Gave talk to the Neurobiology Department at the University of Alabama at Birmingham. "NMDA-receptor independent LTP in hippocampus."
01/28/91	Gave talk on the research in my laboratory to Baylor MD/PhD students
02/04/91– 02/07/91	National Science Foundation, Graduate Fellowship Program Panel, Washington, DC. Reviewed NSF fellowship applications.

7 New Discoveries, Inventions, or Patent Applications

None.

8 References

COLLINGRIDGE, G. L., KEHL, S. J., AND MCLENNAN, H. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol. (Lond.)* 334: 33–46, 1983.

FISHER, R. AND JOHNSTON, D. Differential modulation of single voltage-gated calcium channels by cholinergic and adrenergic agonists in adult hippocampal neurons. *J. Neurophysiol.* 64: 1291–1302, 1990.

FISHER, R. E., GRAY, R., AND JOHNSTON, D. Properties and distribution of single voltage-gated calcium channels in adult hippocampal neurons. *J. Neurophysiol.* 64: 91–104, 1990.

GANNON, R. L., BATY, L. T., AND TERRIAN, D. M. L(+)-2-amino-4-phosphonobutyrate inhibits the release of both glutamate and dynorphin from guinea pig but not rat hippocampal mossy fiber synaptosomes. *Brain Res.* 495: 151–155, 1989.

GANNON, R. L. AND TERRIAN, D. M. BOAA selectively enhances L-glutamate release from guinea pig hippocampal mossy fiber synaptosomes. *Neurosci. Lett.* 107: 289–294, 1989.

GANNON, R. L. AND TERRIAN, D. M., Presynaptic modulation of glutamate and dynorphin release by excitatory amino acids in the guinea pig hippocampus., *(submitted)*, 1990.

GRAY, R. AND JOHNSTON, D. Multiple types of calcium channels in acutely exposed neurons from the adult guinea pig hippocampus. *J. Gen. Physiol.* 88: 25a–26a, 1986.

HARRIS, E. W. AND COTMAN, C. W. Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl D-aspartate antagonists. *Neurosci. Lett.* 70: 132–137, 1986.

HOPKINS, W. F. AND JOHNSTON, D. Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus. *J. Neurophysiol.* 59: 667–687, 1988.

JAFFE, D. AND JOHNSTON, D. The induction of long-term potentiation at hippocampal mossy fibers follows a Hebbian rule. *J. Neurophysiol.* 64: 948–960, 1990.

KORN, S. J. AND HORN, R. Influence of sodium-calcium exchange on calcium current rundown and the duration of calcium-dependent chloride currents in pituitary cells, studied with whole cell and perforated patch recording. *J. Gen. Physiol.* 94: 789–812, 1989.

LLINÁS, R. R. The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. *Science* 242: 1654–1664, 1988.

MACDERMOTT, A. B., MAYER, M. L., WESTBROOK, G. L., SMITH, S. J., AND BARKER, J. L. NMDA-receptor activation increases cytoplasmic calcium concentrations in cultured spinal cord neurones. *Nature* 321: 519–522, 1986.

MALENKA, R. C., KAUER, J. A., ZUCKER, R. S., AND NICOLL, R. A. Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. *Science* 242: 81–84, 1988.

MILLER, R. J. Multiple calcium channels and neuronal function. *Science* 235: 46–52, 1987.

MONAGHAN, D. T. AND COTMAN, C. W. Distribution of *N*-methyl-D-aspartate-sensitive L-[^{3}H]glutamate-binding sites in rat brain. *J. Neurosci.* 5: 2909–2919, 1985.

TERRIAN, D. M., DORMAN, R. V., AND GANNON, R. L., Displacement of endogenous glutamate with D-aspartate: an effective strategy for isolating the calcium-dependent component of release from synaptosomes., *(submitted)*, 1990.

TERRIAN, D. M., GANNON, R. L., AND REA, M. A. Glutamate is the endogenous amino acid selectively released by rat hippocampal mossy fiber synaptosomes concomitant with prodynorphin-derived peptides. *Neurochem. Res.* 15: 1–5, 1990.

WILLIAMS, S. AND JOHNSTON, D. Muscarinic depression of long-term potentiation in CA3 hippocampal neurons. *Science* 242: 84–87, 1988.

WILLIAMS, S. AND JOHNSTON, D. Long-term potentiation of hippocampal mossy fiber synapses is blocked by postsynaptic injection of calcium chelators. *Neuron* 3: 583–588, 1989.